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Participation of two human cytomegalovirus immediate early gene regions in transcriptional activation of adenovirus promoters.

Tevethia MJ, Spector DJ, Leisure KM, Stinski MF.

Department of Microbiology, College of Medicine, Pennsylvania State University, Hershey 17033.

The participation of human cytomegalovirus (HCMV) immediate early genes in the activation of the expression of adenovirus genes in trans (trans-activation) was examined. The initial strategy used was to determine the ability of HCMV genes to complement mutants of adenovirus E1a, an immediate early gene which encodes a trans-activator. The HCMV immediate early gene regions IE1 and IE2 complemented E1a-deficient mutants in three separate assays. IE1 and IE2 substituted for E1a in the synthesis of infectious adenovirus, late adenovirus RNA, and adenovirus DNA. Complementation by the IE2 gene region alone, but not by IE1 alone, was observed using the most discriminating assay, that for late adenovirus RNA synthesis. A role for both HCMV gene regions in positive transcriptional control was indicated by their ability to increase expression of chloramphenicol acetyltransferase (CAT) mediated by the adenovirus E2a promoter. The IE2 region alone activated CAT synthesis but IE1 alone had no detectable activity. Moreover, the activity of both gene regions was about 10-fold higher than that of IE2 alone. These data indicate that efficient complementation of E1a-deficient mutants and trans-activation of adenovirus early promoters involved the participation of both HCMV immediate early gene regions.

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Identification and characterization of the human cytomegalovirus immediate-early region 2 gene that stimulates gene expression from an inducible promoter.

Hermiston TW, Malone CL, Witte PR, Stinski MF.

The human cytomegalovirus (HCMV) XbaI E cloned DNA fragment of approximately 20 kilobases can complement an adenovirus mutant (dl312) defective in the E1a viral gene product (D. J. Spector and M. J. Tevethia, Virology 151:329-338, 1986). This viral DNA fragment contains three immediate-early (IE) genes between 0.709 and 0.751 map units (M. F. Stinski, D. R. Thomsen, R. M. Stenberg, and L. C. Goldstein, J. Virol. 46:1-14, 1983). Two of the IE genes, IE1 and IE2, were isolated and tested for a role in regulating viral gene expression. Since HCMV early and late promoters require additional characterization, the chloramphenicol acetyl transferase (cat) gene, driven by the adenovirus E2 promoter, was used as an indicator of gene expression. cat expression from this heterologous viral promoter was shown to be stimulated by HCMV at early times after infection. The IE1 gene product did not function independently in activating this promoter. The IE2 gene products could independently stimulate the expression of a plasmid of a plasmid when the cat gene was placed downstream of the inducible E2 promoter (E2CAT). Five proteins of different sizes have been predicted to originate from IE2, depending on mRNA splicing. The protein products specified by the IE2 gene were characterized with an antibody to a synthetic peptide according to the open reading frame of exon 2. Three of the five proteins are encoded by exon 2. Three viral proteins of 82, 54, and 28 kilodaltons (kDa) were detected. The exons contained in the region designated as IE2a have open reading frames that could code for two of the smaller proteins of 27 and 30 kDa. This region, when driven by the HCMV enhancer, could independently stimulate gene expression from E2CAT to a high level. A plasmid with the HCMV enhancer upstream of exons, that could code for the HCMV IE2 proteins of 48 and 51 kDa, as well as 27- and 30-kDa proteins, also stimulated E2CAT expression but at a lower level. The activity of this plasmid was augmented by the IE1 gene product, despite the fact that the latter gene product alone was inactive. It is proposed that the HCMV IE region 2 gene products are involved in the regulation of viral or host cell promoters either independently or in combination with other HCMV IE proteins.

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